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Notes

Toxicity of waters from the St. Lawrence River at Massena Area-of-Concern to the plankton species *Selenastrum capricornutum* and *Ceriodaphnia dubia*

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ABSTRACT

In 1972, the US and Canada committed to restore the chemical, physical, and biological integrity of the Great Lakes Ecosystem under the first Great Lakes Water Quality Agreement. During subsequent amendments, part of the St. Lawrence River at Massena NY, and segments of three tributaries, were designated as one Area of Concern (AOC) due to various beneficial use impairments (BUIs). Plankton beneficial use was designated impaired within this AOC because phytoplankton and zooplankton population data were unavailable or needed "further assessment". Contaminated sediments from industrial waste disposal have been largely remediated, thus, the plankton BUI may currently be obsolete. The St. Lawrence River at Massena AOC remedial action plan established two criteria which may be used to assess the plankton BUI; the second states that, "in the absence of community structure data, plankton bioassays confirm no toxicity impact in ambient waters". This study was implemented during 2011 to determine whether this criterion was achieved. Acute toxicity and chronic toxicity of local waters were quantified seasonally using standardized bioassays with green alga *Selenastrum capricornutum* and water flea *Ceriodaphnia dubia* to test the hypothesis that waters from sites within the AOC were no more toxic than were waters from adjacent reference sites. The results of univariate and multivariate analyses confirm that ambient waters from most AOC sites (and seasons) were not toxic to both species. Assuming both test species represent natural plankton assemblages, the quality of surface waters throughout most of this AOC should not seriously impair the health of resident plankton communities.

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Introduction

During the 1970s and 80s, the governments of Canada and the United Stated committed to restoring the physical, chemical, and biological integrity of Areas of Concern (AOC) throughout the Great Lakes under the Great Lakes Water Quality Agreement (GLWQA) (http://www.epa.gov/greatlakes/glwqa/1978/index.html). An AOC is "a geographic area that fails to meet the general or specific objectives of the agreement where such failure has caused or is likely to cause impairment of beneficial uses or of the area's ability to support aquatic life." Part of the St. Lawrence River (and lower reaches of the Grass, St. Regis, and Raquette Rivers) at Massena, New York (Fig. 1) was designated as one of 43 AOCs with likely or known impairment to eight beneficial uses caused mainly by past industrial pollution (NYSDEC, 1990). The "degradation of phytoplankton and zooplankton populations" or the plankton Beneficial Use Impairment (BUI) was designated as "unknown" and in need of further assessment, in the St. Lawrence River at Massena AOC because plankton community information was unavailable. Results of a recent investigation suggest that plankton communities within much of the system may be relatively healthy (Twiss et al., 2010), hence, the plankton BUI may be outdated in all, or parts of, this AOC.

A primary goal of the St. Lawrence River at Massena (and Cornwall) Remedial Action Plan (RAP) is to "restore, protect, and maintain the chemical, physical, and biological integrity of the St. Lawrence River ecosystem and in particular the Akwesasne, Cornwall-Lake St. Francis and Massena Area of Concern in accordance with the Great Lakes Water Quality Agreement and other agency laws, regulations, and policies" (Hartig and Thomas, 1988; NYSDEC, 1990, 2006). The RAP established specific criteria in the St. Lawrence River at Massena AOC for removing the "Degradation of Phytoplankton and Zooplankton Populations" BUI. These criteria are: (1) "When phytoplankton and zooplankton community structure does not significantly diverge from unimpacted control sites of comparable physical and chemical characteristics", and (2) "Further, in the absence of community structure

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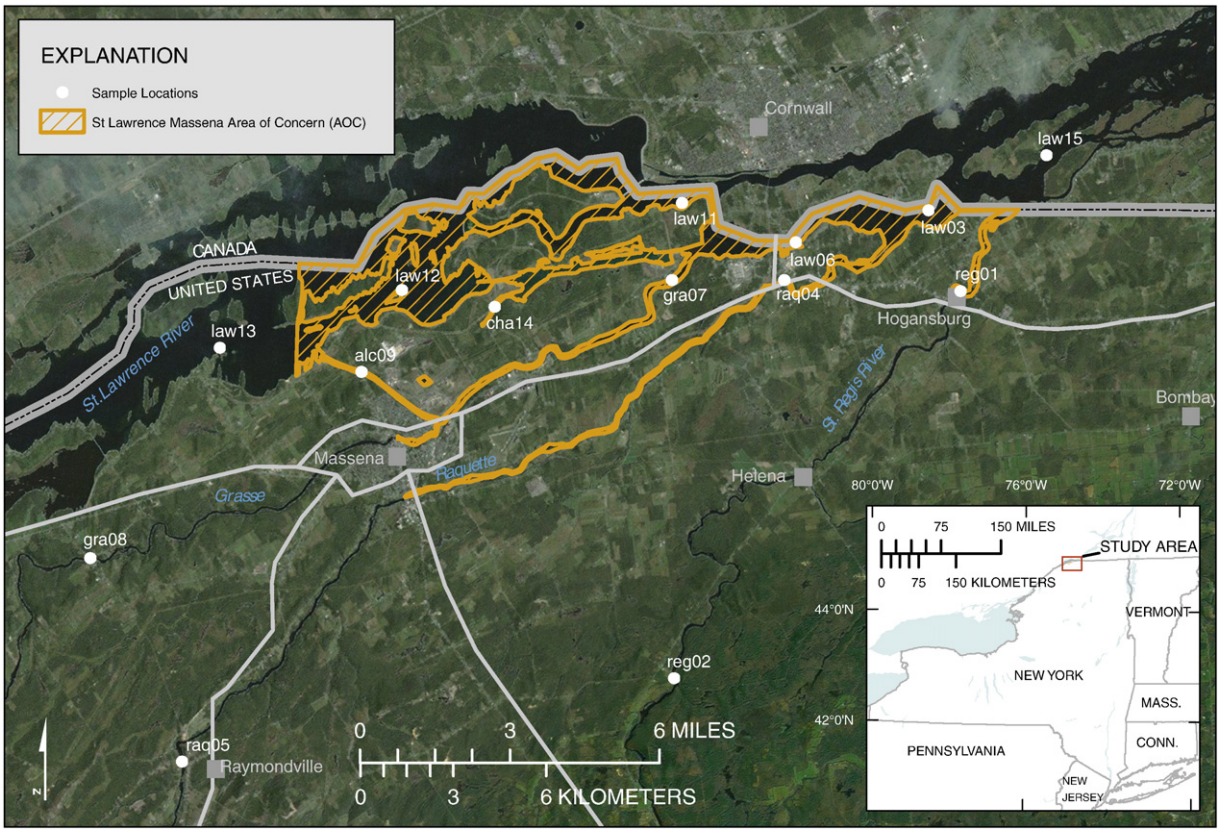

Fig. 1. Map of the St. Lawrence River, major tributaries, sampling sites, and the boundaries of the St. Lawrence River at Massena NY AOC.

data, this use will be considered restored when phytoplankton and zooplankton bioassays confirm no significant toxicity in ambient waters" (George and Boyd, 2007; IJC, 1991).

Historically, high concentrations of nutrients (nitrogen and phosphorus) and dense algal blooms led to the designation of "Eutrophication or Undesirable Algae" as another BUI within this AOC. Recent reductions in nutrient inputs due to control actions in combined sewer outflow and nonpoint sources (and possibly the colonization of the St. Lawrence by zebra mussels) have improved water quality and suggest that the associated beneficial use goal is now being met and maintained (NYSDEC, 2006). Since the nutrient (eutrophication) issue has abated within the St. Lawrence River at Massena AOC, any confounding effects of nutrients on toxicity (of AOC waters to both test species) should be nominal. Therefore, nutrients are not directly evaluated by the present investigation; permitting the focus to remain on the question of toxicity.

In 2011, the U.S. Geological Survey, the St. Regis Mohawk Tribe, and the American Aquatic Testing initiated a study to assess the toxicity of ambient waters from across the St. Lawrence River at Massena AOC and determine if the plankton BUI could be removed in this AOC. This investigation focused only on the second plankton BUI delisting criterion because the composition of riverine phytoplankton and zooplankton assemblages can vary considerably both temporally and spatially (Basu et al., 2000a,b; Hudon, 2000; Reynolds, 2000; Twiss et al., 2010). This high variability is an important limitation for removing BUIs (George and Boyd, 2007); it frequently makes many of the quantitative metrics, needed to characterize biological communities and to test for site-to-site differences, difficult to generate precisely (Stemberger et al., 2001). Seasonal (spring, summer, fall) plankton toxicity data were gathered through 2011 and used to test the hypothesis that waters from selected sample locations within the AOC were no more toxic to two plankton species than were waters from control reaches (in three main tributaries and in the St. Lawrence River) which were not located within the AOC. Both species, the green alga *Selenastrum capricornutum* and water flea *Ceriodaphnia dubia* are very sensitive to toxins and are widely distributed in ponds, marshes, and lakes across most of the United States and Canada (USEPA, 2002a; WDNR, 2004). The synonymy for *S. capricornutum* indicates that *Pseudokirchnerella subcapitata* is currently the accepted taxonomic nomenclature (http://www.fritschalgae.info/Selenastrum.html), however, the present study refers to the species as *S. capricornutum* to remain consistent with toxicity literature. Standardized toxicity tests (bioassays) have been developed for both species, and are commonly used to assess the levels of nutrients or toxins in freshwater environments (USEPA, 2002b). The results from chronic toxicity tests that exposed both plankton species to waters from nine AOC and five control sites are summarized below.

Methods

A total of 45 water samples from nine sites within the AOC and five control sites outside the AOC (Fig. 1) were collected during May, August, and October 2011 and used to quantify potential toxicity of these whole waters to the green algae species, *S. capricornutum* and to the water flea *C. dubia*. There were only two basic criteria for all study site locals; they had to be either within the AOC or outside the AOC (George and Boyd, 2007; IJC, 1991). Thus, sites within the AOC were selected so they encompassed one or more potential upstream contaminant sources, and were generally near the downstream of AOC boundaries for the three tributary rivers and sometimes upstream and downstream of the three river confluences within the St Lawrence River. This design maximized the likelihood of capturing contaminated waters from minimally diluted sources within the three tributaries and from the tributaries within the St. Lawrence. Since the reference sites only needed to be outside the AOC, they were selected where overhead access to the center of river channels was possible. One upstream reference site

was sampled for each river, and an additional downstream reference site was sampled in the St. Lawrence. Sample locations consisted of two sites in the Grass River, two sites within the Raquette River, two sites on the St. Regis River, six sites on the St. Lawrence, one site in the power channel near the ALCOA plant, and one site in a side channel between the upper and lower seaway locks (Table 1). Both of the power and side channel sites (within the AOC) were included because the resultant data were expected to characterize toxicity in potentially unique segments of the St. Lawrence AOC. One duplicate water grab sample was collected from one study site during each of the three seasons to assess the quality (precision) of data generated from the toxicity tests. In general, each sample was collected in the middle of the channel (or the AOC in the St. Lawrence) and at mid-depth from a cableway, bridge, or boat using clean Teflon® tubes and a 12-V DC pump. The low flows which occurred during August 2011 permitted several samples to be collected at most tributary control (and some AOC) sites by hand while wading. At each site, 2 L of water was placed into two 1-L polyethylene containers, stored on ice, and shipped to the testing laboratory (American Aquatic Testing) on the same day that they were collected. Upon arrival at the laboratory, all water samples were filtered to 0.45 μm (USEPA, 2002b) to remove indigenous organisms which may compete with test organisms and confound results. Sample information (site identification code, site name, depth, temperature, collection time, name of field person, and method) were recorded on a field log and chain-of-custody form (that accompanied sample shipments) when each sample was collected.

American Aquatic Testing initiated all toxicity tests within 24 h of collection following standardized U.S. Environmental Protection Agency (USEPA) Test Methods 1003 and 1002 (USEPA, 2002b). In general, the potential toxicity of each AOC (and control) site water sample was assessed using one phytoplankton species, *S. capricornutum* and one zooplankton species, *C. dubia*. Both analyses used chronic endpoints as primary indicators of any adverse response. For *S. capricornutum*, a four replicate static 96-h exposure was used for all samples utilizing a 10,000 cells/mL initial loading and continuous illumination (86 $\mu E/m^2/s$) at 25 °C for the test duration. At the end of the exposure period, cell counts were performed via spectrophotometer on all replicates at each site and a single average generated for comparisons to the control samples. Any sample found with statistically fewer total cells at the end of the exposure period would be considered adversely impacted. Complete guidelines for maintaining *S. capricornutum* cultures, conducting growth tests, and interpreting results are available in Section 14, Table 3 of USEPA Method 1003 (USEPA, 2002b). For *C. dubia*, a seven-day (daily water renewal) exposure with ten individuals (each treated as a separate replicate) was used for all samples. These tests utilized a 16-h light and 8-h dark light cycle, daily renewal of food, and daily observations of survival and production of offspring (young). Any sample found with statistically fewer surviving adults or statistically fewer offspring (total number produced by all adults at the end of the exposure period) would be considered adversely impacted. The specific guidelines for handling organisms, conducting *C. dubia* tests, and interpreting reproduction and survival results are provided in Section 13, Table 3 of USEPA Method 1002 (USEPA, 2002b).

Bioassay data were summarized and used, mainly in univariate analyses, to assess the statistical significance of differences between mean or median *C. dubia* survival and reproduction and algal-cell production (toxicity endpoints) as determined at the end of respective exposures to waters from AOC sites and corresponding upstream non-AOC (control) sites using the Statgraphics® centurion XVI software (StatPoint, 2010). The differences for all one-sided statistical tests were considered significant at $\alpha = 0.05$ ($P \leq 0.05$). A Bonferroni correction (Hochberg, 1988) would have changed α to 0.0005 for all univariate tests, but it was not applied to our analyses to more conservatively categorize site-to-site differences. In brief, Shapiro–Wilk's tests were used to evaluate whether the growth and reproduction (chronic) data from each control and AOC site were normally distributed. A Students *t*-test or one-factor ANOVA was used to assess the significance of differences in mean chronic responses among each AOC site and their upstream control site if data were normally distributed (all but a single algae test) and if a Bartlett's test for homogeneous variance confirmed that the standard deviations for the responses being assessed (at paired sites) were not significantly different. These steps evaluate the statistical differences in endpoints from paired toxicity tests (using control and AOC waters) and correspond to the procedures endorsed in Appendix H of USEPA (2002b) for assessing a "single concentration toxicity test". When mean or median toxicity from one or more sites was found to be significantly different from those at the control site, the 95% confidence intervals were examined graphically and Fisher's Least Significant Difference (LSD) procedure was used to identify which AOC site(s) had higher toxicity than that found in waters from control site(s). A Kruskal–Wallis one-way analysis of variance test, and 95% confidence intervals, were used to assess the significance of the differences in median toxicity between sites if the responses were not normally distributed and (or) if the variances of data pairs were found to be significantly different (more than half of the *C. dubia* tests). Kruskal–Wallis tests were also used to assess differences in median (acute) *C. dubia* survival data because the responses for each adult (replicate) at the end of all tests were binary (either survived = 1 or not survived = 0) and were not normally distributed. The non-parametric Kruskal–Wallis test not only is basically equivalent to a

Table 1
Site name, ID, type, and location (latitude and longitude — NAD83) for toxicity-test samples collected during May, August, and October, 2011.

Site name	Site ID	Type	May		August		October	
			Latitude	Longitude	Latitude	Longitude	Latitude	Longitude
St Regis River 01	reg01	AOC	44 58 33.2	74 39 39.9	44 58 25.4	74 39 22.6	44 58 34.3	74 39 25.5
St Regis River 02	reg02	Control	44 51 49.2	74 46 43.6	44 51 49.2	74 46 43.6	44 51 49.2	74 46 43.6
St Lawrence River 03	law03	AOC	44 59 57.9	74 40 26.5	44 59 52.0	74 40 14.8	45 00 00.5	74 40 20.0
Raquette River 04	raq04	AOC	44 58 45.5	74 43 59.1	44 58 45.5	74 43 59.1	44 58 45.5	74 43 59.1
Raquette River 05	raq05	Control	44 50 22.7	75 58 47.4	44 50 22.7	75 58 47.4	44 50 22.7	75 58 47.4
St Lawrence River 06	law06	AOC	44 59 24.7	74 43 42.4	44 59 50.1	74 43 19.2	44 59 42.0	74 43 22.2
Grass River 07	gra07	AOC	44 58 45.7	74 46 44.5	44 58 42.4	74 46 53.4	44 58 44.7	74 46 53.2
Grass River 08	gra08	Control	44 53 55.6	75 01 02.2	44 53 55.6	75 01 02.2	44 53 55.6	75 01 02.2
Alcoa power channel 09	alc09	AOC	44 57 10.4	74 54 22.6	44 57 10.4	74 54 22.6	44 57 10.4	74 54 22.6
St Lawrence River 11	law11	AOC	45 00 06.4	74 46 30.1	45 00 07.9	74 46 33.1	45 00 09.8	74 46 30.3
St Lawrence River 12[a]	law12	AOC	44 58 35.9	74 53 22.7	44 58 33.0	74 52 22.7	44 59 00.2	74 52 05.4
St Lawrence River 13[a]	law13	Control	44 57 35.8	74 57 51.6	44 57 41.4	74 57 30.9	44 57 28.4	74 56 35.8
St Lawrence side channel 14	cha14	AOC	44 58 18.1	74 51 06.2	44 58 18.1	74 51 06.2	44 58 18.1	74 51 06.2
St Lawrence River 15	law15	Control	45 00 55.0	74 37 31.7	45 01 50.4	74 36 23.2	45 01 47.4	74 36 20.8

[a] Latitude and longitude were approximated during the first (May) sample collection.

Table 2
Mean and median density and statistics for the number of *Selenastrum capricornutum* cells produced (density) after four days of exposure to waters from laboratory, control, and AOC sites during chronic growth tests done during May, August, and October 2011. The *P*-values are listed for the Shapiro–Wilk's tests for normality (each site), Bartlett's test for homogeneity of variance (between paired sites), and for parametric or non-parametric tests assessing the significance of differences between the mean (ANOVA) or median (Kruskal–Wallis) number of cells produced (4 replicates) in waters from control sites and associated AOC sites in each river system. [Groups of two to nine sites within the same river system are shaded alike to illustrate the comparisons made between specific control and their associated AOC sites; duplicate samples are denoted with a "d" in the site ID; and na = not appropriate.].

			Statistics for number of cells produced				Test P-value			
System	Sample–site ID	Site type	Mean	Median	SD	SE	Shapiro–Wilk's	Bartlett's	ANOVA	Kruskal–Wallis
					May 17, 2011					
Laboratory	lab	Control	4,473,250	4,588,000	860,446	430,223	0.5189	na	na	na
St. Regis	reg02	Control	2,666,500	2,686,500	942,413	471,207	0.9303	na	na	na
River	reg01	AOC	3,291,500	3,262,500	404,400	202,200	0.7942	0.1982	0.2686	na
Raquette	raq05	Control	3,594,000	3,595,000	96,478	48,239	0.4876	na	na	na
River	raq04	AOC	3,076,750	3,085,500	522,036	261,018	0.9867	0.0214	na	0.2482
Grass	gra08	Control	3,029,500	3,135,000	807,913	403,957	0.8206	na	na	na
River	gra07	AOC	2,852,000	2,660,500	596,550	298,275	0.4158	0.6293	0.7358	na
	law13	Control	3,894,500	3,772,000	407,589	203,795	0.4213	na	na	na
	law15	Control	4,188,250	4,175,000	422,576	211,288	0.3748	0.9538	0.3556	na
	cha14	AOC	4,081,500	4,071,000	480,367	240,183	0.1727	0.7927	0.5744	na
St. Lawrence	cha14d	AOC	3,755,750	3,671,000	665,440	332,720	0.4447	0.4406	0.7343	na
River	alc09	AOC	4,053,500	3,846,000	574,278	287,139	0.2949	0.5861	0.6674	na
	law03	AOC	4,190,000	4,170,000	202,822	101,411	0.8691	0.2810	0.2419	na
	law06	AOC	3,794,750	3,819,000	338,189	169,094	0.7020	0.7654	0.7194	na
	law11	AOC	4,118,500	4,235,000	471,699	235,849	0.6021	0.8151	0.4994	na
	law12	AOC	3,601,500	3,543,000	400,040	200,020	0.7312	0.9761	0.3444	na
					August 1, 2011					
Laboratory	lab	Control	4,583,000	4,571,500	346,157	173,078	0.9050	na	na	na
St. Regis	reg02	Control	4,268,750	4,386,000	321,821	160,911	0.2772	na	na	na
River	reg01	AOC	4,059,500	4,013,000	540,381	270,191	0.6956	0.4159	0.5305	na
Raquette	raq05	Control	3,199,750	3,291,500	309,804	154,902	0.4796	na	na	na
River	raq05d	Control	3,915,750	4,057,000	731,510	365,755	0.6076	0.192	0.1215	na
	raq04	AOC	3,226,000	3,187,000	862,396	431,198	0.2199	0.1273	0.9562	na
Grass	gra08	Control	3,914,000	3,898,500	276,450	138,225	0.6718	na	na	na
River	gra07	AOC	4,524,250	4,862,000	1,014,946	507,473	0.3870	0.0617	0.2900	na
	law13	Control	5,354,250	5,371,500	358,833	179,416	0.9521	na	na	na
	law15	Control	4,951,750	4,835,000	714,738	357,369	0.5590	0.2867	0.353	na
	cha14	AOC	2,045,750	2,038,000	523,967	261,983	0.9817	0.5485	<0.0001	na
St. Lawrence	alc09	AOC	4,885,750	4,630,000	932,168	466,084	0.4126	0.1516	0.3844	na
River	law03	AOC	4,513,500	4,726,000	501,543	250,771	0.0732	0.5947	0.0343	na
	law06	AOC	4,913,250	4,959,500	528,425	264,212	0.7182	0.5398	0.2166	na
	law11	AOC	5,163,750	5,238,500	278,324	139,162	0.5377	0.6853	0.4336	na
	law12	AOC	5,367,000	5,339,500	296,712	148,356	0.2475	0.7612	0.9581	na
					October 17, 2011					
Laboratory	lab	Control	3,471,000	3,494,500	241,704	120,852	0.5881	na	na	na
St. Regis	reg02	Control	3,870,250	3,858,500	307,400	153,700	0.7003	na	na	na
River	reg01	AOC	3,239,750	3,274,500	364,404	182,202	0.7753	0.7855	0.0383	na
Raquette	raq05	Control	3,801,500	3,769,000	362,706	181,353	0.6245	na	na	na
River	raq04	AOC	3,504,750	3,550,500	172,432	86,216	0.4231	0.2529	0.1899	na
	raq04d	AOC	4,077,000	3,925,000	408,928	204,464	0.2636	0.8477	0.3523	na
Grass	gra08	Control	3,558,250	3,525,500	335,127	167,564	0.9111	na	na	na
River	gra07	AOC	3,673,750	3,718,000	229,647	114,823	0.5363	0.5491	0.5903	na
	law13	Control	3,652,250	3,681,000	205,662	102,831	0.8262	na	na	na
	law15	Control	3,961,000	3,943,500	222,856	111,428	0.9392	0.8976	0.0879	na
	cha14	AOC	3,679,500	3,681,000	118,700	59,350	0.7273	0.3895	0.8261	na
St. Lawrence	alc09	AOC	3,612,500	3,599,000	472,396	236,198	0.7793	0.2053	0.8824	na
River	law03	AOC	4,093,500	4,077,000	206,292	103,146	0.4990	0.9961	0.0231	na
	law06	AOC	3,967,750	3,891,000	253,809	126,904	0.4796	0.7368	0.1016	na
	law11	AOC	3,713,750	3,695,500	136,035	68,018	0.8028	0.5133	0.6357	na
	law12	AOC	4,488,500	4,501,500	251,858	125,929	0.6541	0.7461	0.0021	na

Mann–Whitney *U* or Wilcoxon rank sum test, but also can evaluate the significance of differences among more than two groups that are not normally distributed or do not have homogeneous variances. The quality of data generated by all toxicity tests was assured by (a) confirming that the sensitivity of test organisms was within normal limits using three standard reference toxicant (SRT) tests; (b) utilizing laboratory-water controls to verify that test conditions and organism responses met minimally acceptable standards; and (c) including three sets of duplicate (sites) tests to determine if the precision of test endpoints was acceptable (USEPA, 2002a,b).

Additional multivariate analyses were used to assess overall or region-wide differences in chronic toxicity of waters at all control and AOC sites and to identify variations that could be associated with river system or sampling season. A parametric three-factor ($2\times3\times4$) ANOVA was used to assess potential interactions and the significance of site type (AOC vs. control), season (May, August, and October), and river system (Grass, Raquette, St. Regis, and St. Lawrence) on the toxicity of ambient waters to *S. capricornutum*. Non-parametric Kruskal–Wallis tests were used to assess the chronic toxicity (to *C. dubia*) of waters from (a) all AOC sites compared to all control sites, (b) all sites among the four river systems, and (c) all sites among the three seasons. Although all significant differences in toxicity were identified, higher toxicity in waters from AOC sites vs. control sites was the primary concern of this investigation.

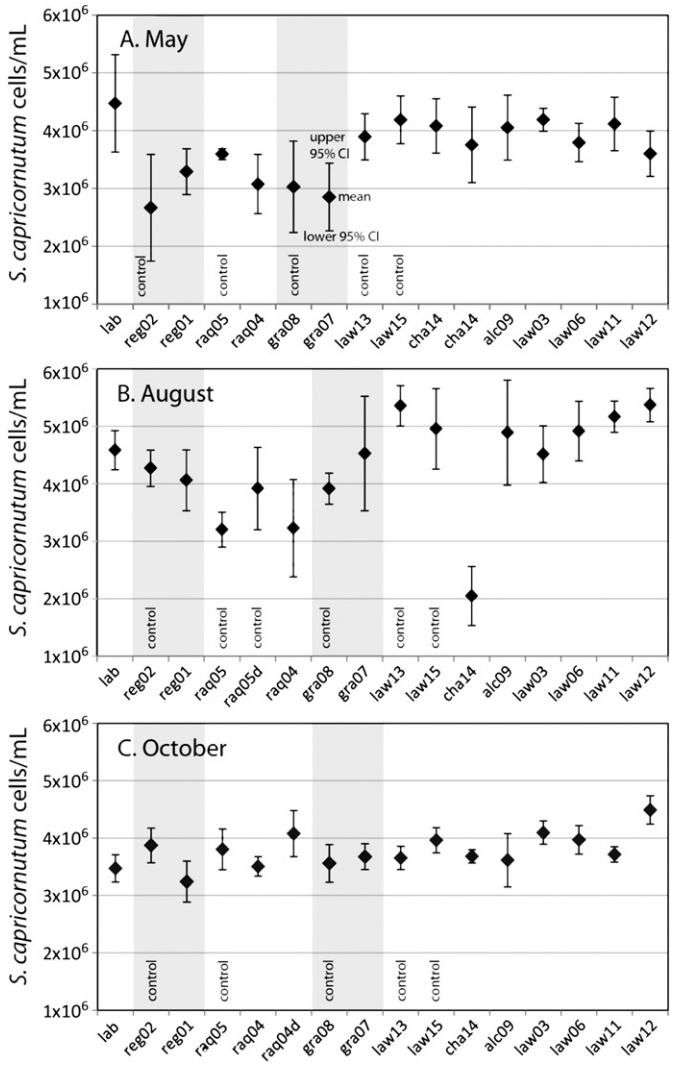

Fig. 2. The 95-percent confidence intervals around the mean density of *S. capricornutum* cells produced at the end of chronic toxicity (growth) tests using waters collected from nine sites in and near the St. Lawrence River at Massena NY AOC and from five control sites on May 17, August 1, and October 17, 2011. [Groups of two to nine sites within the same river system are shaded alike to illustrate the comparisons made between specific control and their associated AOC sites.].

Results

Mean density of *S. capricornutum* ranged from 2.67 to 4.19, 2.05 to 5.37, and 3.24 to 4.49×10^6 cells/mL at the end of tests conducted with waters collected at AOC and control sites during May, August, and October 2011, respectively (Table 2). The production of *S. capricornutum* in the three laboratory controls ranged from 3.47 to 4.58×10^6 cells/mL (Table 2). The results of these laboratory control tests only demonstrate that growth rates were acceptable under the current conditions and that results from these exposures (bioassays) are valid; they are not used for any other statistical comparisons (USEPA, 2002b). All Shapiro–Wilk's tests confirm that the growth data from all sites and test periods were normally distributed and all but one Bartlett's test confirmed that variances were homogeneous for all but one comparison (between control site raq05 and AOC site raq04 during May) (Table 2). Thus, one-factor ANOVA tests (essentially a *t*-test when data from only two sites were assessed) were used for all but the single analysis. Results from the ANOVAs (and the single Kruskal–Wallis test) indicate that, except for sites reg01 (October) and cha14 and law03 (August), mean (or median) densities at AOC sites were either significantly higher (e.g., law03, $P=0.0231$ and law12, $P=0.0021$ during October) or not significantly different from cell densities at their associated control sites (Table 2, Fig. 2). No significant differences were evident during May, but mean cell densities during August were lower at cha14 than at the control site law13 ($P<0.0001$) and densities from law03 were lower than those at law13 ($P=0.0343$), yet densities at law03 were not lower than those at the lower control site law15 ($P=0.3542$; not shown). During October, mean densities were lower at reg01 than at reg02 ($P=0.0383$). The mean number of cells produced in waters from all St. Lawrence and tributary sites was generally highest during the August tests compared to the May and October tests (Fig. 2). The means and 95-percent confidence intervals for density of *S. capricornutum* cells (4 replicates), in waters from each study site after four days of exposure (Fig. 2) clearly show that cell densities in waters from most sites and test periods (except cha14, law03, and reg01 during one month) were similar or higher than that observed in waters from corresponding control sites.

The survival of *C. dubia* during exposure to waters from all AOC and control sites ranged from 70 to 100% (Table 3). The *P*-values for all paired Kruskal–Wallis tests of median *C. dubia* survival were greater than 0.2543 (not shown), and indicate that there were no significant differences in their survival in waters from AOC and corresponding control sites. The mean number of offspring produced by *C. dubia* in waters from all study sites (AOC and controls) ranged from 19.1 to 27.8, 30.3 to 38.2, and 30.7 to 37.7 during chronic tests done in May, August, and October 2011, respectively (Table 3). Results for Shapiro–Wilk's and Bartlett's tests show that data from most sites and test periods were not normally distributed or that the variances were not homogeneous for several comparisons (Table 3). Thus, non-parametric Kruskal–Wallis tests of medians were used in most (18) statistical analyses; 1-factor ANOVAs were used in 11 comparisons. Except for cha14 during October, the results from almost all ANOVA and Kruskal–Wallis tests indicate that the mean or median number of offspring produced by mature females at AOC sites did not differ significantly from the number produced at their associated control sites; 28 of the 29 *P*-values ranged from 0.0560 to 0.9698 (Table 3). The median number of offspring produced in waters from cha14 during October was significantly higher than at the upstream control site law13 ($P=0.0303$, shown), but it was not significantly different from the number produced at the downstream control site law15 ($P=0.3031$, not shown). The means and 95-percent confidence intervals for the number of offspring produced by 10 adults from each study site during 7 days (Fig. 3) clearly show that there were no obvious adverse effects of AOC waters on *C. dubia* reproduction. The mean number of offspring produced in waters from all St. Lawrence and tributary sites was generally lowest during the May tests compared to the August and October tests (Fig. 3).

The number of replicates and statistical power can be increased by combining all data (pooled across all sites) through a multivariate analysis of growth or reproduction observed for each plankton species. Results from a parametric three-factor ANOVA indicate that the density of *S. capricornutum* cells produced in waters from sites in the AOC in general was not significantly different from the densities produced in waters from control sites ($P=0.4526$) and that both river and season had significant effects on density (both *P* values are<0.0001). Although river and season interacted with each other ($P=0.0027$), neither interacted significantly with site type (*P* ranged from 0.4799 to 0.5872). These results confirm that the production of *S. capricornutum* cells in waters from AOC sites generally did not differ from that in waters from control sites, but that cell production within both AOC and control sites sometimes differed between seasons and rivers. Although a multi-factor ANOVA was not usable for assessing pooled *C. dubia* data across all sites, successive one-factor (nonparametric) Kruskal–Wallis tests evaluated differences in the median number of offspring produced at all (pooled) sites between the two types (AOC vs. control), the four rivers, and the three seasons. The results from these analyses indicate that site type, river system, and

Table 3
Mean percent survival and statistics for the number of offspring produced by *Ceriodaphnia dubia* during seven-day exposures to waters from laboratory, control, and AOC sites during chronic-toxicity tests during May, August, and October 2011. The *P*-values for the Shapiro-Wilk's tests for data normality (within each site), Bartlett's test for homogeneity of variance (between paired sites), and parametric or non-parametric tests describing the significance of any differences between the mean (ANOVA) or median (Kruskal–Wallis) number of offspring produced by 10 individuals (replicates) in waters from control sites and associated AOC sites in each river system. [Groups of two to nine sites within the same river system are shaded alike to illustrate the comparisons made between specific control and their associated AOC sites; duplicate samples are denoted with a "d" in the site ID; and na = not appropriate.].

				Statistics for offspring production				Test P-value			
System	Sample-site ID	Site type	Percent survival	Mean	Median	SD	SE	Shapiro–Wilk's	Bartlett's	ANOVA	Kruskal–Wallis
					May 17, 2011						
Laboratory	lab	Control	100	34.7	36.0	7.09	2.241	0.1123	na	na	na
St. Regis	reg02	Control	80	23.3	30.0	12.17	3.847	0.7289	na	na	na
River	reg01	AOC	100	27.0	27.0	7.56	2.390	0.1632	0.8778	0.8817	na
Raquette	raq05	Control	80	25.4	32.5	14.27	4.512	0.0206	na	na	na
River	raq04	AOC	90	26.3	29.0	11.11	3.512	0.0510	0.4666	na	0.6492
Grass	gra08	Control	90	27.8	32.5	11.48	3.630	0.0108	na	na	na
River	gra07	AOC	70	26.9	35.5	17.02	5.382	0.0111	0.2560	na	0.3829
	law13	Control	70	24.9	29.5	11.51	3.641	0.0579	na	na	na
	law15	Control	80	25.7	29.0	11.05	3.493	0.0480	0.9038	na	0.9698
	cha14	AOC	90	25.7	27.0	9.90	3.131	0.0623	0.6600	0.8695	na
St. Lawrence	cha14d	AOC	80	22.7	26.5	9.49	3.000	0.0247	0.0573	na	0.2549
River	alc09	AOC	70	19.1	23.5	13.19	4.173	0.0787	0.6910	0.3088	na
	law03	AOC	80	26.6	31.0	12.39	3.919	0.0208	0.8296	na	0.5959
	law06	AOC	80	25.6	29.0	11.82	3.736	0.1039	0.9396	0.8947	na
	law11	AOC	70	23.6	30.0	14.20	4.490	0.0027	0.5418	na	0.9697
	law12	AOC	80	24.3	30.0	16.15	5.108	0.0269	0.3273	na	0.9698
					August 1, 2011						
Laboratory	lab	Control	100	33.7	34.5	6.40	2.022	0.1008	na	na	na
St. Regis	reg02	Control	100	38.2	39.0	4.18	1.323	0.8398	na	na	na
River	reg01	AOC	100	34.3	34.0	7.45	2.357	0.1228	0.1005	0.1663	na
Raquette	raq05	Control	95	30.8	34.0	11.33	3.583	0.0002	na	na	na
River	raq05d	Control	100	35.1	35.5	3.25	1.027	0.8037	<0.0001	na	0.5409
	raq04	AOC	100	33.3	34.0	2.58	0.817	0.3891	0.0002	na	0.7314
Grass	gra08	Control	100	35.7	38.0	7.45	2.357	0.0017	na	na	na
River	gra07	AOC	100	35.6	37.0	3.84	1.213	0.2211	0.0608	na	0.4894
	law13	Control	100	31.3	32.5	5.03	1.592	0.3789	na	na	na
	law15	Control	100	32.0	31.0	7.06	2.231	0.9442	0.3289	0.8013	na
St. Lawrence	cha14	AOC	100	35.9	37.0	5.51	1.741	0.2380	0.7936	0.067	na
River	alc09	AOC	100	33.5	36.0	7.71	2.437	0.5506	0.2205	0.4596	na
	law03	AOC	100	32.2	33.0	3.91	1.236	0.5636	0.4629	0.6606	na
	law06	AOC	100	32.4	32.5	2.99	0.945	0.7037	0.1352	0.5598	na
	law11	AOC	100	30.3	33.0	8.33	2.633	0.0326	0.1498	na	0.7903
	law12	AOC	100	31.0	32.0	7.27	2.300	0.1331	0.2881	0.9158	na
					October 17, 2011						
Laboratory	lab	Control	100	30.1	35.5	10.49	3.318	0.0521	na	na	na
St. Regis	reg02	Control	90	33.0	36.5	12.84	4.061	0.0059	na	na	na
River	reg01	AOC	100	36.2	38.5	8.32	2.632	<0.0001	0.2125	na	0.2543
Raquette	raq05	Control	90	34.8	35.5	7.07	2.235	0.0828	na	na	na
River	raq04	AOC	100	32.2	33.5	3.97	1.254	0.0270	0.1004	na	0.1475
	raq04d	AOC	100	30.7	31.5	8.42	2.663	0.1994	0.6101	0.2536	na
Grass	gra08	Control	100	33.5	35.5	7.50	2.372	0.0028	na	na	na
River	gra07	AOC	100	33.1	35.0	6.66	2.105	0.0024	0.7276	na	0.7009
	law13	Control	100	33.1	35.5	5.63	1.779	0.0241	na	na	na
	law15	Control	100	36.0	36.0	2.91	0.919	0.3220	0.0621	na	0.4016
	cha14	AOC	100	37.5	38.5	2.37	0.749	0.0673	0.0168	na	0.0303
St. Lawrence	alc09	AOC	100	37.7	38.0	3.74	1.184	0.1956	0.2404	na	0.0560
River	law03	AOC	100	33.3	36.0	7.79	2.463	0.0711	0.3463	na	0.7610
	law06	AOC	100	31.6	36.0	12.65	4.001	0.0396	0.0243	na	0.6763
	law11	AOC	100	35.5	37.5	7.01	2.217	0.0014	0.5220	na	0.1831
	law12	AOC	100	35.5	36.0	3.92	1.241	0.9173	0.2974	na	0.4469

the various sites did not significantly affect the number of *C. dubia* offspring produced ($P = 0.4596$, 0.0624, and 0.6323, respectively), but season did affect offspring production ($P < 0.0001$). Although growth and offspring production differed among rivers and seasons (see Figs. 2, 3), these multivariate findings, combined with results from the individual (univariate) comparisons, indicate that waters from most study sites within the AOC were generally not toxic to the two plankton species during the three sampling periods.

The quality of data generated by all toxicity tests was assured by evaluating the sensitivity of test organisms and the results from laboratory-water controls and duplicate samples. The 7-d, 25% inhibition concentration (IC_{25}) values generated from three SRT tests ranged from 336.0 to 368.4 ppm KCl for *C. dubia*; the 96-h IC_{25} values for the *S. capricornutum* SRT tests ranged from 958.2 to 1876.9 ppm KCl. All IC_{25} values fell within the normal ranges for each test organism. For all laboratory-control exposures, *C. dubia* survival exceeded 80%; at least 60% of surviving *C. dubia* produced a third brood within seven days; 15 or more offspring were produced per surviving female; and the mean density of *S. capricornutum* exceeded 1×10^6 cells/mL and the variability in density was less than 20% (mean CV = 11.1%) for all replicates after 96 h. Data from the three sets of duplicate showed that the absolute Relative Percent Difference (RPD) for *C. dubia* survival, *C. dubia* production, and *S. capricornutum* growth averaged 5.6, 11.1, and 14.5%, respectively.

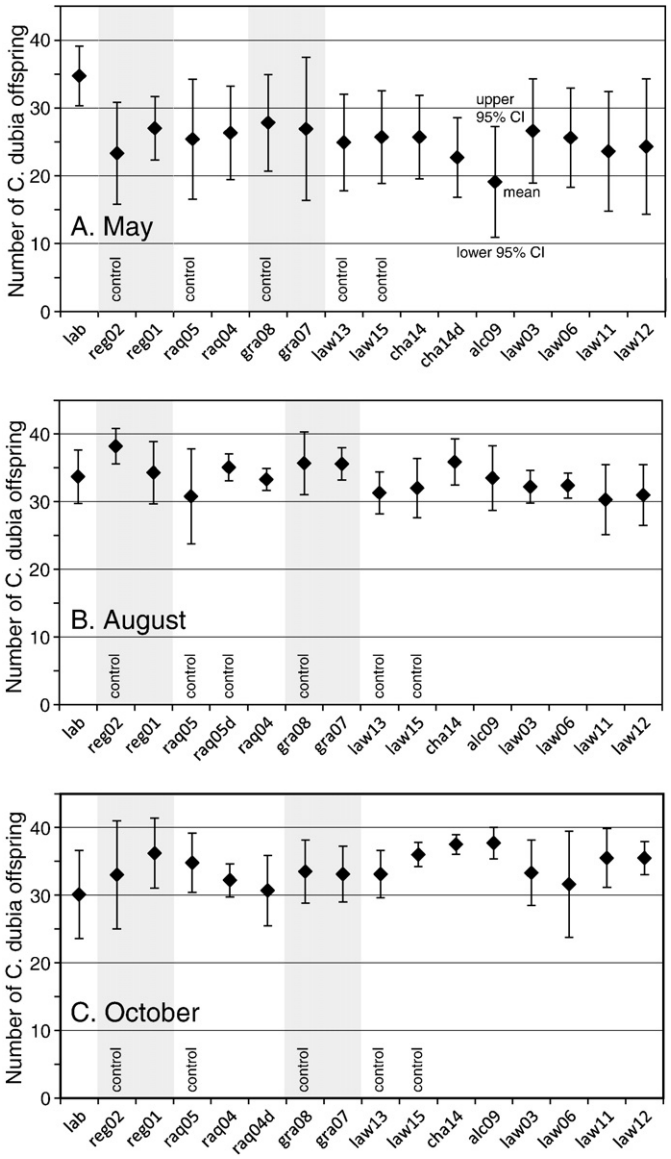

Fig. 3. The 95-percent confidence intervals around the mean number of *C. dubia* offspring produced at the end of 7-d chronic (reproduction) toxicity tests using waters collected from nine sites in and near the St. Lawrence River at Massena NY AOC and from five control sites on May 17, August 1, and October 17, 2011. [Groups of two to nine sites within the same river system are shaded alike to illustrate the comparisons made between specific control and their associated AOC sites.].

Discussion

With only a few exceptions, the bioassay results demonstrate that ambient waters across the St. Lawrence at Massena AOC were generally not acutely or chronically toxic to the two species of plankton evaluated by this study. Survival data from the *C. dubia* tests showed that whole waters from all AOC (and control) sites were not acutely toxic to this zooplankton species. In addition, the number of *C. dubia* offspring produced in waters from all AOC sites was consistently comparable to the number of offspring produced in waters from corresponding control sites. The results of growth tests with the phytoplankton species *S. capricornutum* also indicated that cell production in waters from AOC sites was not significantly different, or was significantly higher, than production in waters from corresponding control sites in 26 of 29 individual comparisons. More compelling evidence was provided by multivariate analyses (using pooled data for both plankton species), which confirmed the view that waters from across the AOC were generally not toxic to either *S. capricornutum* or *C. dubia*. These analyses essentially demonstrated that water quality at almost all sites within the St. Lawrence River (and tributaries) at Massena AOC were comparable, or at least, that waters from AOC sites were no more toxic to the two plankton species than were waters from upstream (and downstream) control sites located outside the AOC. Provided that several assumptions are true (see below) and study limitations are tolerable, the findings largely support the thesis that waters in the St. Lawrence River at Massena AOC are currently not toxic to local plankton species and their populations.

The sampling design, bioassay procedures, and resultant toxicity data for this study were unavoidably limited, which could potentially subject our findings and conclusions to certain criticisms. The few (two) test species, the number of sites (spatial extent), and the number of sampling events (periods) were restricted mainly by the number of toxicity tests (total of approximately 45 tests for each species) that could be funded. Nevertheless, one might question whether the two species are representative of, or will respond similarly to, the native phytoplankton and zooplankton communities. Though the concern is valid, both species are widely distributed in North America (including the Great Lakes Region) and are very sensitive to a wide range of contaminants and nutrients (USEPA, 2002b). Just as important, the USEPA has used both species as universal plant and invertebrate surrogates for decades in standardized tests used to quantify biota responses to acute or chronic toxicity in wastewaters and natural streams (USEPA, 2002b). Consequently, few other species would be as effective in detecting impaired water quality in the St. Lawrence River as *C. dubia* and *S. capricornutum*. The ability of ethylenediaminetetraacetic acid (EDTA) to sequester or chelate metal ions, and its use as a standard ingredient in micronutrient stock solutions for maintaining algal cultures, may also cause *S. capricornutum* growth tests to underestimate toxicity that could be linked to metals. Past interlaboratory comparisons, however, showed that one-third of growth tests generated false positives (incorrectly detected toxicity) and that test variability was nearly doubled when conducted without EDTA (USEPA, 2002a). Because EDTA substantially improves test method performance, there are few options other than including it in stock solutions used during *S. capricornutum* tests. In addition, the three sample seasons were selected to target collection periods when small boats could safely operate on the St. Lawrence and when water quality conditions might vary with extremes in temperature and flow. During water collections at reg02 (USGS station 04269000, St Regis River at Brasher Center, NY), daily mean discharge was 8380, 495, and 2220 ft^3/s and water temperature was 16.5, 23.8, and 10.6 °C, on May 17, August 1, and October 17, 2011, respectively. The annual mean discharge for this station from 1910 to 2010 was 1064 ft^3/s (http://wdr.water.usgs.gov/wy2010/pdfs/04269000.2010.pdf). These data illustrate that river conditions during the three sampling periods varied considerably, and encompassed a broad range in river flows which normally occur each year. The total number of sample sites (given three sampling events) was also moderately limited (total of 14 sites) (Fig. 1). By definition control sites only had to be located outside of the AOC, therefore, one was selected upstream of the AOC in each tributary, and two were selected in the St. Lawrence River — one upstream and one downstream of the AOC. One control site would technically meet test and BUI-removal requirements, thus, the five control sites were more than adequate. Only one AOC site was selected within each tributary because the water quality at downstream reaches should integrate conditions at upstream reaches. Due to its three tributaries, channel complexity, and the length of the St. Lawrence River segment within the AOC (approximately 24 km), four AOC sites were targeted within the river proper, a fifth AOC site (cha14) was situated within a backwater between the upper and lower Seaway locks, and a sixth AOC site (alc09) was located in the "power channel" linking the St. Lawrence River and the Raquette River. The number of AOC sites in the St. Lawrence was considered

acceptable because their locations permitted water quality to be characterized both upstream and downstream of tributaries which could either dilute or increase contaminant concentrations within the main channel. In general, the sampling design was deemed sufficient to effectively characterize the spatial and temporal variability in water quality and the differences in potential toxicity of waters across the St. Lawrence River at Massena New York AOC as well as in the lower reaches of the three tributaries that fall within the boundaries of this AOC.

The significantly lower density of *S. capricornutum* cells in waters from site reg01 during October (and at sites law03 and cha14 during August) suggests that the waters at these three AOC sites could be seasonally toxic. These results do not necessarily contradict the contention that waters across most of the AOC are generally not toxic to plankton for several reasons. First, the same waters were not toxic to the Cladoceran *C. dubia* during the same sampling periods. Although sensitivities typically vary between species, the absence of toxicity of any AOC site waters to *C. dubia* suggests that toxicants may not be the primary issue. Second, waters from site cha14, which had large reductions in density of *S. capricornutum* cells during August, occupy a relatively unique position within the St. Lawrence River system, and may not truly represent water-quality conditions which exist across the AOC. The site is a relatively stagnant backwater marsh located off of the main shipping channel between the upper and lower locks on the Seaway (Fig. 1). Water-surface elevations often change, and flows reverse, at this site when large volumes of water are released from the nearby upper lock. These conditions may produce unique water–sediment interactions and nutrient cycling, which could affect phytoplankton growth, survival, and reproduction. Lastly, the small number of AOC sites that were found to be significantly different (more or less toxic) from the controls did not consider the effects of random chance (error) on the numerous statistical tests. Univariate analyses clearly show that toxicity of waters to *S. capricornutum* was significantly lower at AOC sites than in corresponding control sites in 3 of 29 comparisons. None of the 29 *C. dubia* univariate tests detected significantly lower offspring production in AOC site waters. Thus, only 3 of 58 (total) paired tests indicated that toxicity was significantly higher in waters from AOC sites than in waters from their associated control site(s). Given the probability of making a type II error (rejecting a true null hypothesis) is 5% when alpha = 0.05, then the 3 significantly toxic findings (5.1% of bioassays) detected by the analyses were comparable to the percentage that would be expected to be significant due to random chance, even if no real differences existed. As a result, the univariate analyses which identified significant toxicity to *S. capricornutum* in three tests could theoretically be attributed to random chance. This possibility is buttressed by the multivariate tests which have more statistical power than univariate (paired site-to-site) tests and indicate that waters from AOC sites generally have no significant toxic effect on the reproduction or growth of the two test species. Despite the speculation above, the substantial and significant reduction in growth of *S. capricornutum* at cha14 during August, and the slight and significant reductions at law03 during August and at reg01 during October, cannot simply be disregarded. The toxic responses noted in waters from these sites should be investigated further to determine if they are spurious findings or if they could be linked to legacy contaminants, related to seasonal changes in ambient water quality, or caused by some unknown factor(s) within the AOC.

Although AOC site waters were significantly toxic to *S. capricornutum* in a few instances, a singular focus on the outcome of statistical analyses could be misleading or even misrepresent expected ecosystem responses to contaminated or mitigated waters within the AOC. The analyses presented herein assess statistically significant differences (or the lack thereof) between toxicity-test endpoints at control and AOC sites, and do not necessarily determine if any observed differences (whether significant or not) are biologically meaningful. The absence of statistically significant differences between toxicity results at control and most AOC sites is buoyed by relatively small changes (some positive) in survival and production of *C. dubia* and the growth of *S. capricornutum* in waters from AOC sites when compared to that observed in waters from respective control sites. Although the percentage differences varied somewhat among river systems, the number of *C. dubia* offspring produced in waters from all AOC sites was on average 0.8% lower (range: −23 to +16%) than that produced in waters from all control sites; *C. dubia* survival in waters from all AOC sites averaged 4.5% higher (range: −22 to +29%) than did survival in waters from all control sites. Except for the 63% lower production in waters from one site (cha14) during August, *S. capricornutum* growth (densities of cells) in waters from all AOC sites averaged 0.7% lower (range: −16 to +23%) than did densities in waters from all control sites. Even when significantly affected at reg01 (October) and law03 (August) sites, the growth of *S. capricornutum* was only 16–18% lower than that at their corresponding control sites (Table 3). The nominal decreases in mean production (less than 1%) for both species and the nearly 5% increase in survival of *C. dubia* support the thesis that most waters across the St. Lawrence River AOC have no meaningful biological effects on resident plankton species and their populations.

The use of bioassays to characterize the quality or toxicity of ambient waters across the Great Lakes is an effective tool for characterizing the capacity of ambient waters to sustain key beneficial uses. This is undoubtedly one reason why plankton bioassays (that confirm no significant toxicity) were identified as an optional criterion for removing plankton BUIs within several AOCs in the Great Lakes along the US and Canadian border (George and Boyd, 2007). Toxicity tests essentially avoid several drawbacks commonly associated with quantifying indigenous biota and testing for significant differences among sites. Site-to-site differences in habitat and water quality; seasonal changes in temperature, daylight, and river flows; and variable recruitment of lentic plankton from upstream sources, create large spatial and temporal fluctuations in density and biomass of plankton species and their communities (Basu et al., 2000a,b; Hudon, 2000). These issues function to make precise characterization of indigenous populations and communities at comparable sites, and statistical assessments, problematic. Toxicity tests simply characterize toxicity, or the lack of toxicity, for waters collected at strategic points within a river network. The primary requirement for toxicity-test water is that it comes from sites situated either upstream (control) or downstream of known point or non-point sources of contaminants (with in the AOC). The plankton community assessments require extensive characterizations of the physical and thermal habitat at control and AOC sites to prove that they are not dissimilar and that habitat variations could not account for site-to-site differences in species populations or community structure. Although acute and chronic toxicity results cannot prove that the lower trophic levels of natural ecosystems are fully intact and healthy, the information provides convincing evidence that both plankton species and their communities should not be adversely affected by impaired water quality at most locations inside and outside of the St. Lawrence River at Massena NY AOC. There is no reason why similar toxicity tests could not be employed to evaluate BUIs for plankton, as well as for other species, in AOCs across the Great Lakes Region.

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